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ORIGINAL PAPER

Cross-fostering by foreign conspecific queens and slave-making workers influences individual- and colony-level personality

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Abstract Recent studies of inter-individual variation in behavior have focused primarily on its environmental causation and adaptive consequences, but commonly ignore questions regarding its proximate development. Here, we explore the effects of the late natal environment on the development of individual- and colony-level personalities in the acorn ant, *Temnothorax longispinosus*. This species is commonly parasitized by the slave-making ant *Protomognathus americanus*, and we predicted that rearing *T. longispinosus* with its brood parasite would alter the behavior of individual ants and the collective, colony-level personality of groups. Using a split-brood design (where brood from a single source colony is split equally across different rearing environments), we reared *T. longispinosus* in four conditions: their maternal queen, an unrelated conspecific queen, a slave-making queen, and a slave-making worker. Although individual aggressiveness and exploratory behavior did not differ between ants raised by their maternal queen or slave-making ants, ants raised by an unrelated conspecific queen showed increased aggressiveness 60 days after emergence. Further, groups of ants raised by slave-making workers were faster at locating new nest sites, a collective behavior, relative to groups reared by their maternal queen. Lastly, colonies containing *T. longispinosus* and their maternal queen had the greatest brood production at 60 days. Our results demonstrate that differences in individuals' rearing environment can influence both individual- and colony-level personality in multi-level societies.

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Introduction

Virtually, any casual observer of animal behavior would note that, within any given species, individuals exhibit characteristic differences in their behavior. Although such individual variation has classically been viewed as statistical noise of little importance, recent years have seen a surge in the number of papers devoted to exploring the causes and consequences of individual variation (referred to in the literature as "behavioral types" or "animal personalities") (Gosling 2001; Dall et al. 2004, 2012; Sih et al. 2004). This recent emphasis across all of ecology (Bolnick et al. 2003, 2011) is due, in part, to new discoveries on the widespread impact of individual variation (particularly in behavior) on multiple levels of ecological and/or social organization. For general ecology, individual differences in behavior can shape individual fitness (Both et al. 2005), species interactions (McGhee et al. 2013), population spread (Cote et al. 2010), and community dynamics (Keiser and Pruitt 2014). For animal societies, variation in behavioral types or personality can occur among social groups, between castes, and even among seemingly egalitarian individuals (Sih et al. 2004; Jandt et al. 2014). And variation at any of these levels of organization could have important consequences for the ecology of social groups.

In social groups, distinct and/or divergent personalities can exist along at least two levels of organization: individuals and groups. Understanding the causal relationships between these two organizational levels of personality is fundamental to our understanding of the evolution, development, and persistence of individual differences in behavior (Pinter-Wollman 2012; Jandt et al. 2014). Despite a few studies linking individual and group personalities (Grinsted et al. 2013; Pruitt et al. 2013;

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Brown and Irving 2014), the field lacks a comprehensive understanding of the causes of this interaction. One notable pitfall of the animal personality literature is that the influence of ontogeny is rarely addressed (Groothuis and Trillmich 2011). Given that there is a rich history detailing the nature of the rearing environment as a determinant of future behaviors (e.g., learning, imprinting, etc.), we argue that the natal social environment could be a key component in the development and emergence of individual and group (i.e., colony-level) personalities. However, studies in the personality literature rarely account for factors of the natal social environments in driving the development of individual behavioral types (but see Sendova-Franks and Franks 1993; Ravary et al. 2007; Niemelä et al. 2012; Sweeney et al. 2013). Parallel studies on colony-level personality are genuinely absent.

Taxa that may be particularly interesting models to test the factors of the developmental environment are those where very young offspring (e.g., brood, fledglings, etc.) interact with their natural enemies in the natal and early developmental environment. For example, early experience with predators and agonistic encounters with conspecifics can alter the course behavioral development in ants and other arthropods (e.g., McDonald and Topoff 1986; Sato and Nagayama 2012). Many ant colonies are also inhabited by inquilines (i.e., social parasites) like Lycaenid caterpillars that compete with or eat host brood (Pierce et al. 2002). Similarly, avian brood parasites compete with or kill host offspring while exploiting adult hosts (Hauber 2003). A special case of brood parasitism—slave-making—exemplifies the exploitation of heterospecific offspring. Slave-making worker ants raid hosts' nests in order to abduct pre-emerged heterospecific brood to exploit as a slave "workforce" in their own nests (Topoff 1990).

Protomognathus americanus, a slave-making ant, is an obligate social parasite where queens are unable to establish their own colonies and therefore must invade host colonies to obtain heterospecific pupae (Herbers and Foitzik 2002; Pamminger et al. 2012). Once these heterospecific pupae develop in the slave-making nest, they will perform all routine tasks for the colony, including foraging and brood care (Herbers and Foitzik 2002). Once colonies are established, *P. americanus* workers and host slaves conduct raids to further increase colony size and their workforce (Brandt et al. 2005a, b). However, a critical stage in the development of *P. americanus* colonies involves only a slave-making queen and her heterospecific slaves (aka the incipient colony; (Pamminger et al. 2012)).

A primary host of *P. americanus* is the acorn ant *Temnothorax longispinosus*, which forms small colonies in preformed cavities in acorns, hickory nuts, and twigs in temperate forests (Headley 1943). This species is very well suited to study how the social environment influences the development of worker personality, because mean worker aggressiveness is consistent across worker generations, indicating that aggressiveness is genetically and/or developmentally determined (Modlmeier et al. 2012). Moreover, more aggressive colonies are better protected against invading slave-making queens resulting in fewer stolen brood (Pamminger et al. 2012). Thus, the usurpation of colonies by invading parasitic queens is a strong selective pressure on worker behavior (Heinze and Keller 2000). Although *T. longispinosus* queens control the behavior of their workers via tactile and chemical mechanisms (Konrad et al. 2012), it is unknown how interactions in the natal social environment influence the development of aggressive phenotypes. How might the development of individual personalities change if broods are raised by a slave-making queen as opposed to their own maternal queen?

Here, we conduct a split-brood design where late-stage *T. longispinosus* worker pupae from a single colony are cross-fostered in one of four rearing environments: alongside a slave-making *P. americanus* queen, a slave-making *P. americanus* worker, an unrelated conspecific queen, or their own maternal queen. We then test the individual personalities of worker ants (aggressiveness and exploratory behavior) at two stages of development and finally measure their collective, colony-level personality across three behaviors (nest discovery, collective foraging, and colony defense).

Specifically, we hypothesize that (I) individual and collective behaviors will differ between workers raised by their own queen vs. any other rearing environment; (II) workers raised by slave-making ants will exhibit greater aggressiveness and exploratory behavior, thus more closely matching the behavioral phenotypes of their "foster parents" which may increase success during raiding events (e.g., Schuett et al. 2013); (III) colonies containing a maternal queen will exhibit the highest brood production, as the phenotypes of these workers and queens will be the most complementary; and (IV) colonies containing workers which are more aggressive and exploratory will perform better at collective behaviors like nest discovery, collective foraging, and colony defense.

Methods

Collection and maintenance

Mature queenright colonies of *T. longispinosus* and *P. americanus* were collected in May 2013 at the Huyck Preserve, Albany County, NY (all colonies ranged in size from 20–40 adult workers). Colonies were transported back to the laboratory at the University of Pittsburgh, where they were maintained in clear plastic boxes (14 cm×13 cm×4 cm) with a plastic inlay artificial nest (7.5 cm×2.5 cm×0.3 cm). Colonies were continuously supplied with water and fed ad libitum each week with honey and crickets, *Acheta*

domesticus. Colonies were maintained at room temperatures (22–25 °C) with a natural light/dark cycle (approximately 11.75 h light:12.25 h dark).

Experimental procedure

Two months after collection, ten *T. longispinosus* colonies containing at least 20 worker pupae close to eclosion were distributed among four different rearing environments in a split-brood design. Five pupae were placed in a new, clean nest box with their own mature maternal queen, an unrelated mature conspecific queen from a foreign site, a mature slave-making *P. americanus* queen, or a mature slave-making *P. americanus* worker. The slave-making worker treatment represents an ecologically relevant environment in which brood could be raised, because some colonies which occur in nature have been found to contain only host ants and one or a few slave-making workers (Herbers and Foitzik 2002). The foreign conspecific queen treatment acts as a control for brood being raised by an unrelated queen (i.e., the slave-making queen). The pupae were assigned to rearing environments randomly and each pupa eclosed within 24 h of placement in the colony. Thus, any developmental effects that we observed were more likely the result of interactions in the early post-eclosion social environment as opposed to developmental effects from maternal care. Ten experimental colonies were created for each of the four rearing environments (n=40 colonies total). All experimental colonies were provided the same ad libitum food and water and maintained in a random array under the same laboratory conditions.

Once all pupae developed into adults, we assessed the behavioral tendencies of individuals (aggressiveness and exploratory behavior) at 5 and 60 days after eclosion and the collective behavior of colonies (nest discovery, collective foraging, and colony defense) at 70 days after eclosion. Sixty days after eclosion is an important time point for *Temnothorax* colonies; this is about the time that colonies start competing for nest sites which become limited in the early fall (Foitzik and Heinze 1998). During this time, collective behaviors like nest relocation and colony defense should be crucial for the colony. Further, we have previously shown that both these behaviors are influenced by the individual personalities of its group members (Modlmeier et al. 2014a). Lastly, we counted the number of brood each queen had produced 60 days after the onset of the experiment. None of these new broods had eclosed throughout the 70 days, so only the original workers were present in the colonies at each assay time point. There was little to no worker mortality in our experimental colonies, and mortality did not differ between treatments ($F_{3,39}$=1.50, p=0.23). To ensure that differences among colonies in their collective behavior were stable, we measured each colony twice in each of our collective behavioral assays. Consecutive measurements were separated by 14 days, and trials were completed in the order presented here.

Individual behavioral assays

Aggressiveness We measured individual aggressiveness after Modlmeier and Foitzik (2011) by placing all workers individually into circular arenas (diameter=12 mm, height=3 mm) covered by a glass slide (n=5 workers/colony, n=200 workers total). After a 60-s acclimation period, a recently frozen and defrosted unrelated *T. longispinosus* worker collected from a site foreign to the focal ant (at least 1.6 km away) was added to the arena. Dead ants were used as opponents in order to negate any behavioral influence an opponent may have on the behavior of the focal ant (Crosland 1990; Roulston et al. 2003), as it has been previously shown that aggressiveness towards dead opponents reflects aggression towards live opponents (Modlmeier and Foitzik 2011). We observed the interactions of each ant with the dead opponent during ten 20-s scans (200 s total). At each time point, we recorded whether the focal ant was interacting "aggressively" (i.e., mandible spreading, biting, dragging, carrying, and stinging) or not (i.e., ignoring the opponent, antennating) to determine the proportion of time in which ants behaved aggressively. Opponents were used no more than three times with different workers, and no worker was tested with the same opponent twice. This methodology has proven effective in previous work, and no effects were observed between the first and third use of the opponent (e.g., Pamminger et al. 2012; Modlmeier and Foitzik 2011; Modlmeier et al. 2012). Arenas were cleaned with isopropanol after each test to remove any odors from previous trials (although odors could potentially remain on the opponent ant). To confirm that individual differences in aggressiveness were repeatable, we measured a subset of individuals (n=60) from 20 different experimental colonies twice per day for two consecutive days at the end of our study. Individuals used to assess repeatability were kept in individually marked 0.5-ml Eppendorf tubes with a piece of damp paper overnight.

Exploratory behavior To test individual exploratory behavior, we placed an individual ant in the center of a multi-chambered assay space after Modlmeier and Foitzik (2011). This space consisted of a central chamber (diameter=29 mm, height=3 mm) connected to eight equally sized empty chambers through corridors (length=32 mm, width=7 mm) radiating symmetrically from the center. We allowed workers to explore the environment freely for 5 min while we recorded the number of novel chambers visited during this time. A chamber was said to have been entered when an ant's entire body cleared the chamber's threshold. We cleaned the assay

chambers with isopropanol before each test and allowed it to evaporate for at least 1 min.

Collective behavioral assays

Nest discovery To assess colonies' latency to discover a new nest following destruction of their original nest, a new artificial nest was placed 3 cm away from and parallel to the colonies' current nest. This distance between old and new nest sites simulate a distance that naturally occurs (*unpublished data* associated with Modlmeier and Foitzik 2011). We removed the top and sidewalls of the current nest and recorded the latency for a worker to discover the new nest (discovery time). We consider this a collective measure of nest discovery, as the detection of the new nest by the first individual is ostensibly the product of a network of interactions between nestmates and characterizes the collective activity and exploratory behavior of the colony (Detrain and Deneubourg 2006). A worker was said to have discovered a new nest when its entire body cleared the nest entrance threshold. Colonies were allowed 120 min to discover the new nest, after which time we terminated the trial.

Collective foraging Colonies were starved for 5 days prior to all foraging assays. Following the starvation period, each colony was provided a fresh, small drop of honey on a standard (7.5 cm×2.5 cm) microscope slide positioned 3 cm away from the nest. We recorded the latency for the first ant to feed (discovery time) as well as the number of ants feeding at the honey every 5 min for 90 min.

Colony defense Colony defense was tested after Scharf et al. (2012) by introducing a recently frozen and defrosted unrelated *T. longispinosus* worker collected from a site foreign to the focal colony (at least 1.6 km away) into the entrance of the artificial nest of the experimental colony. We then blocked the entrance of the colony with a piece of plastic and used 20-s scan sampling to record all aggressive (mandible spreading, biting, dragging, carrying, and stinging) and nonaggressive (antennating, ignoring) actions towards the opponent for 5 min. All aggressive acts were summed for each colony, and we used this number as our measure of colony-level aggressiveness.

Statistical analyses

Values representing individual exploratory behavior were log transformed, and individual aggressiveness data were arcsine square root transformed to meet model assumptions. Behavior at the individual worker level was analyzed using a nested mixed-effect model with natal environment as an independent variable and natal environment nested in brood source colony identity as a random effect. We used generalized linear models (GLM) with the identity link function to analyze how brood source colony identity, natal environment, mean aggressive, and exploratory behavior at day 60 influence collective behaviors. All variables were normally distributed (Kolmogorov-Smirnov test: $p>0.10$), except for mean aggressive behavior, which was normalized with a square root transformation. We used q values to control for the false discovery rate resulting from multiple testing. These values represent the proportion of false positives among the significant results, and the p value should be lower than the q value to remain significant (Storey 2002). All collective behavioral assays were performed twice with 14 days between trials to calculate repeatability. The mean value of the two colony-level trials was then used for all subsequent analyses.

We used a model selection approach using the Akaike information criterion corrected for small sample sizes (AICc) to identity the best subset of predictor variables for each collective behavior, including natal environment, mean colony aggressiveness, mean colony exploration, and brood source identity (Johnson and Omland 2004). Here again, we used q values to account for the possibility of type I error from multiple testing. We performed all possible variable combinations (excluding interactions) during the model selection procedure (15 total models). Repeatability was estimated using GLMM with colony ID or individual ID as a random effect. The proportion of the variation explained by colony ID/individual ID was our estimate of repeatability. We tested for relationships between individual behaviors with linear regression analysis and between collective behaviors with nonparametric Spearman's correlations. The individual-level statistical analyses were performed in JMP version 10 (SAS Institute Inc., Cary, NC, USA), and the collective behaviors were analyzed with Statistica 9.1 (StatSoft Inc., Tulsa, Oklahoma, USA).

Results

Individual behavioral assays

Aggressiveness The aggressiveness of individual workers was highly repeatable ($F_{50,180}=1.70$, $p=0.006$, repeatability= 0.64). At 5 days after emergence, mean aggressiveness of workers did not differ between any rearing environment ($F_{3,36}=0.47$, $p=0.77$). However, at 60 days after emergence, mean aggressiveness of workers that were raised by a foreign conspecific queen was greater than that of brood raised in any other rearing environment ($F_{3,36}=3.24$, $p=0.03$; $q=0.08$, Fig. 1).

Exploratory behavior At both 5 and 60 days after emergence, there was no difference in exploratory behavior between ants

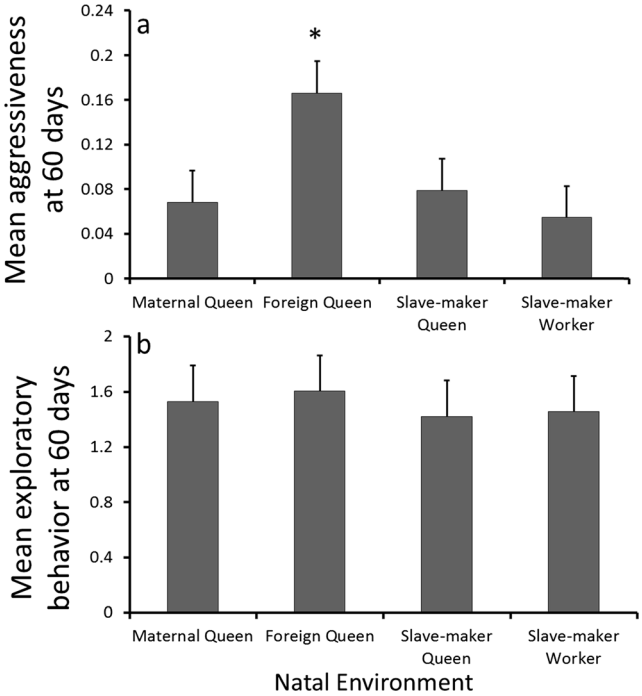

Fig. 1 At 60 days after emergence, **a** mean aggressiveness was greatest in workers raised by an unrelated conspecific queen ($F_{3,36}$=4.24, p=0.03, q=0.08). However, at this time point, **b** mean exploratory behavior did not differ between individual ants raised across all four rearing environments ($F_{3,36}$=1.59, p=0.21). Values significantly different from the others are denoted with an *asterisk*

raised in any of the rearing environments ($F_{3,36}$=1.59, p=0.21; Fig. 1).

Individual-level syndrome We failed to detect a significant correlation between aggressiveness and exploratory behavior in any of our rearing environments (all $p \geq 0.19$), nor were they significant after 60 days (all $p \geq 0.62$).

Brood source identity The variance component estimates from the random effect test suggest that the identity of the source colony from which the brood originated influenced both the aggressiveness and exploratory behavior of individual workers (Table 1). Brood source colony has a stronger effect on exploratory behavior than aggressiveness at both 5 and 60 days. Further, the identity of the brood source colony had a significant effect on collective nest discovery, where workers collected from source colonies 2, 5, and 9 differed significantly in their collective behavior from other source colonies (Table 2).

Collective behavioral assays

Nest discovery The best models according to the AICc are presented in Table 2. The AICc scores for all 15 models are available in Online Resource 1. Here, we discuss the three best models, because both their AICc values (Δ $AICc_{1-2}$=0.1 and Δ $AICc_{1-3}$=0.4) and their Akaike weights (0.36, 0.34, and 0.30) were extraordinarily similar and the corresponding metrics of the fourth model decreased precipitously. All three models found a significant effect of natal environment where, compared to the maternal queen treatment, being raised by a *P. americanus* worker augmented nest discovery (i.e., decreased the time needed to discover a new nest; Table 2; Fig. 2a). In addition, the first and second models found a significant effect of brood source identity, i.e., workers from some source colonies were faster in nest discovery than workers from other source colonies and vice versa (Table 2). The third model did not include brood source, but showed that mean worker aggressiveness at day 60 after emergence improved nest discovery time (Fig. 2b). Further, this was not driven by the traits of the most aggressive individual (GLM: Wald stat.=0.76563, p=0.38). Nest discovery time was consistent at the colony level across two assays (repeatability=0.36; Spearman's ρ=0.44; p=0.004); however, multiple incidences of maximum values in the data set could have influenced this correlation. Significance was lost when data points containing at least one maximum value were removed (Spearman's ρ=0.32; p=0.13).

Collective foraging Foraging at the colony level could not be significantly explained by the predictors in any models (all p>0.13, repeatability=0.11).

Colony defense Colony-level aggressiveness could not be significantly explained by the predictors in any model (all p>0.25). However, colony defense values were highly repeatable across two assays (repeatability=0.24, d.f.=39, p=0.001). Lastly, we did not find any correlation between mean worker aggression at day 60 and colony-level total aggression (ANCOVA: $F_{1,39}$=1.5142, p=0.22).

Colony-level syndrome We detected significant correlations in collective behavior across contexts in two of our four natal environments. That is, no collective behaviors were correlated at the colony level for workers raised by their maternal queen (all $p \geq 0.31$) or slave-making queens (all $p \geq 0.21$). However, in colonies where workers were raised by an unrelated conspecific queen, colony defense and collective foraging were negatively correlated (Spearman's ρ=−0.69, p=0.03, q=0.286). That is, under this developmental environment, groups which exhibited more pronounced collective defensive behavior needed less time to discover a new food source. In colonies where workers were raised by slave-making workers, collective foraging and nest discovery were positively correlated (Spearman's ρ=0.68, p=0.029, q=0.286). Thus, groups which discovered new nest sites faster also discovered food sources faster.

Brood production After 60 days, colonies containing a *T. longispinosus* queen and her own worker offspring had

Table 1 Restricted maximum likelihood estimates of variance components of natal environment nested in brood source colony

Time point behavioral assay	Variance component	Standard error	95 % confidence intervals	Significant effect
5 days				
Aggressiveness	0.0279	0.0066	0.02, 0.05	Yes
Exploratory behavior	0.2817	0.0664	0.19, 0.48	Yes
60 days				
Aggressiveness	0.0279	0.0066	0.02, 0.05	Yes
Exploratory behavior	0.3557	0.0850	0.23, 0.61	Yes

the most new brood produced in their nests ($F_{3,36}$=4.37, p=0.01; Fig. 3).

Discussion

Here, we show that the rearing environment can be a driver of both individual- and colony-level personalities in a temperate ant, but that personality divergence is not readily observable early in life. As the literature on animal personality expands, the interest in understanding the emergence and maintenance of personality at multiple levels of biological organization (i.e., individuals, social groups, populations) grows. To date, few studies have linked individual- and colony-level personalities, and fewer yet have measured this relationship in a developmental framework (Stamps and Groothuis 2010; Groothuis and Trillmich 2011; Niemelä et al. 2012; Sweeney et al. 2013).

Individual behaviors

At 5 days after emergence, there were no differences in the mean aggressiveness or the mean exploratory behavior of worker ants raised by a slave-making queen, a slave-making worker, an unrelated conspecific queen, or their own maternal queen. This time point may have preceded a critical or sensitive period where behavioral types diverge to match current environmental conditions (Bischof 2007; Groothuis and Trillmich 2011) which may suggest either a limitation to or cost of early behavioral plasticity (Estramil et al. 2014). Selection may favor developmental plasticity at certain early life stages where the adaptive value of adult behaviors depends on the characteristics of the juvenile environment (West-Eberhard 2003). Further, the source colony from which ants originated had a strong influence on individual behavior across all rearing environments. This indicates a strong genetic component underlying exploration and aggressiveness, which corroborates previous studies on this species (Modlmeier et al. 2012, 2014a, b).

At 60 days after emergence, however, mean exploratory behavior remained the same across all rearing environments, but mean aggressiveness was greatest in workers raised by unrelated conspecific queens. In fact, workers reared by foreign conspecific queens were 2.5 times more aggressive than those reared in any other environment (Fig. 1). Relatedness between queens and workers can facilitate more complimentary phenotypes and can be crucial to the maintenance of cooperation in ant colonies (Perez-Uribe et al. 2003), whereas a lack of relatedness might produce phenotypic mismatching within these colonies, potentially leading to the observed increase in worker aggressiveness (Linksvayer 2008). Cross-fostering brood by queens from different colonies can shift colony sex ratios, a sign of queen-worker reproductive conflict (Passera et al. 2001), and cross-fostering by workers from alternative reproductive social backgrounds can distress growth rates and survival (Purcell and Chapuisat 2012). It has also been suggested, however, that the role of queens in regulating the development of worker behavior is less important in highly developed eusocial Hymenoptera (e.g., ants; Robinson 1992). Thus, the relative roles of queen-worker relatedness and other environmental factors on the development of individual behaviors should be investigated further.

Even more surprising is that the developmental trajectory of worker behavior did not differ between brood reared by their maternal queen or by a slave-making ant, their principal natural enemy. This potentially demonstrates the tightness of the coevolutionary arms race between *P. americanus* and *T. longispinosus*, in that the parasites can so closely intercept the nestmate recognition mechanisms of their host that they fail to alter the host's personality (Brandt et al. 2005a, b, 2006). Although it has been hypothesized that some slave-making ants mark their hosts with nest-specific hydrocarbon profiles (Alloway and Keough 1990), *P. americanus* seems rather to adjust the relative quantities of cuticular chemicals already present on the pupae they rear (Brandt et al. 2005a, b). Similarity in the cuticular hydrocarbon profiles between slave-making ants and their hosts is a principal mechanism by which nestmate recognition is intercepted (Brandt et al. 2006; Errard et al. 2006; but see Liu et al. 2003). Thus, *P. americanus* might be able to intercept the nestmate recognition system of its host

Table 2 Parameter estimates for the three best generalized linear model (GLM) for nest discovery according to AICc

Effect	Level of effect	Estimate	S.E.	Wald stat.	*p* value
Model 1[a]					
Intercept		*4111.11*	*527.210*	*60.80672*	*0.000000*
Natal environment	Slave-making worker	*−1373.86*	*442.466*	*9.64109*	*0.001903*
Natal environment	Foreign queen	852.42	490.664	3.01813	0.082338
Natal environment	Slave-making queen	−317.66	427.411	0.55237	0.457350
Brood source	Source colony 1	−200.93	777.397	0.06681	0.796044
Brood source	Source colony 2	*2658.48*	*751.274*	*12.52192*	*0.000402*
Brood source	Source colony 3	377.31	739.717	0.26017	0.610004
Brood source	Source colony 4	370.53	746.616	0.24629	0.619700
Brood source	Source colony 5	*−2073.64*	*739.778*	*7.85710*	*0.005062*
Brood source	Source colony 6	156.79	744.187	0.04439	0.833137
Brood source	Source colony 7	−270.83	744.180	0.13244	0.715913
Brood source	Source colony 8	824.91	748.767	1.21372	0.270596
Brood source	Source colony 9	*−1494.92*	*746.275*	*4.01272*	*0.045158*
Mean Aggression		−2969.00	1856.002	2.55895	0.109671
Model 2[b]					
Intercept		*3365.66*	*254.3277*	*175.1273*	*0.000000*
Natal environment	Slave-making worker	*−1188.71*	*440.5084*	*7.2819*	*0.006965*
Natal environment	Foreign queen	465.94	440.5084	1.1188	0.290180
Natal environment	Slave-making queen	−289.96	440.5084	0.4333	0.510381
Brood source	Source colony 1	−583.54	762.9830	0.5849	0.444384
Brood source	Source colony 2	*2868.71*	*762.9830*	*14.1366*	*0.000170*
Brood source	Source colony 3	387.46	762.9830	0.2579	0.611575
Brood source	Source colony 4	532.84	762.9830	0.4877	0.484952
Brood source	Source colony 5	*−2091.91*	*762.9830*	*7.5172*	*0.006111*
Brood source	Source colony 6	287.46	762.9830	0.1419	0.706351
Brood source	Source colony 7	−401.41	762.9830	0.2768	0.598812
Brood source	Source colony 8	638.96	762.9830	0.7013	0.402338
Brood source	Source colony 9	*−1653.16*	*762.9830*	*4.6946*	*0.030257*
Model 3[c]					
Intercept		*4391.19*	*606.065*	*52.49609*	*0.000000*
Natal environment	Slave-making worker	*−1443.43*	*555.062*	*6.76251*	*0.009309*
Natal environment	Foreign queen	997.63	603.364	2.73388	0.098240
Natal environment	Slave-making queen	−328.07	540.185	0.36884	0.543638
Mean aggression		*−4084.52*	*2070.201*	*3.89276*	*0.048495*

All estimates for the natal environments describe the parameter effect compared to the maternal queen treatment. The *q* values and whole-model *p* values for each of the three models are as follows: model 1: $p=0.008$, $q=0.03$; model 2: $p=0.01$, $q=0.03$; model 3: $p=0.05$, $q=0.05$. Significant values are set in italics

[a] Model 1: AICc=730.3463; Δ AICc=0; Akaike weight=0.36

[b] Model 2: AICc=730.4844; Δ AICc=0.1381; Akaike weight=0.34

[c] Model 3: AICc=730.7518; Δ AICc=0.4055; Akaike weight=0.30

by merely altering the ratios of various key cuticular hydrocarbons.

Collective behaviors

Our results demonstrate a diversity of subtle and frequently counterintuitive consequences of rearing environment on collective personality. When compared to brood raised by their own queen, ants raised by a *P. americanus* worker needed less time to discover a new nest, while workers raised by a foreign conspecific queen required more time. Furthermore, workers raised by a *P. americanus* queen did not differ from those raised by their own queen in their collective nest discovery. Colonies whose workers were more aggressive discovered new nests

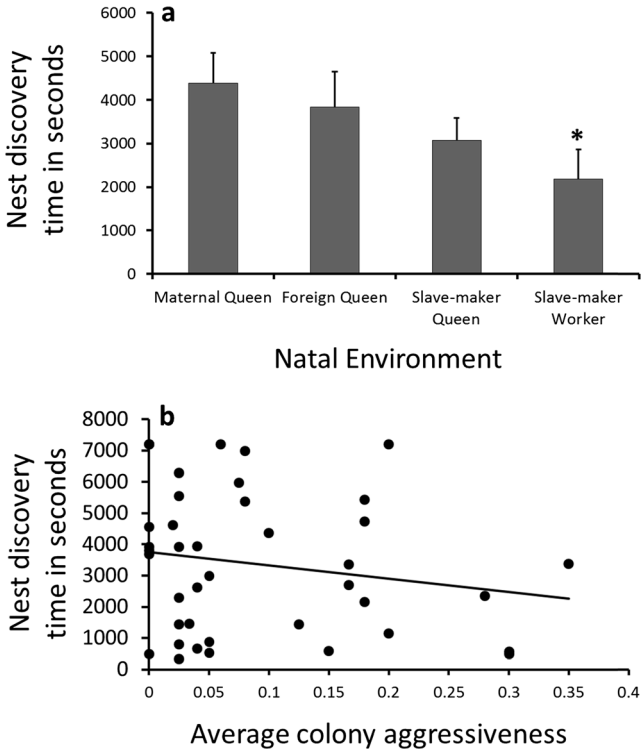

Fig. 2 The time required to discover a new nest after their original natal nest was destroyed and was influenced by the natal environment (**a**) and decreased with the average aggressiveness of workers in the colony (**b**) (GLM parameter estimates, β=−1.33, p=0.03, q=0.03). Colonies raised by a slave-making worker were faster in collective nest discovery. Values significantly different from the maternal queen treatment are denoted with an *asterisk*

faster after their natal nest was destroyed, and this trend was not driven by the traits of the most aggressive individual. Thus, collective nest discovery, across all social contexts, is not driven by the behavior of a single "keystone individual" (Modlmeier et al. 2014a, b) as seen in social spiders (Pruitt and Keiser 2014). Although, the latency for an individual within a colony to discover a new nest is likely the result of a combination of individual and collective behavioral traits. After accounting for colony aggressiveness, the *P. americanus* worker treatment had the greatest positive influence on the nest discovery efficiency of its workers. Although the foreign conspecific queen treatment resulted in higher worker aggressiveness (which improves discovery efficiency), these colonies were not as efficient as they should have been, given their aggressiveness. This seemingly counterintuitive observation suggests that there is some other, unidentified factor about either (i) these rearing environments or (ii) the source colonies from which workers were collected that supersedes mere aggressiveness for determining nest discovery time. Alternatively, being raised by a *P. americanus* worker may have had the greatest influence on collective nest discovery because "queenless" colonies often develop aggressiveness-based dominance hierarchies and marked shifts in behavior (e.g., Heinze and Oberstadt 1999; Clémencet et al. 2008; Konrad et al. 2012). This, in turn, might explain why workers were faster to identify new nest sites. A future test of this hypothesis could be to rear *T. longispinosus* workers in the absence of a queen and compare their nest discovery time to those exhibited by our slave-making worker treatment.

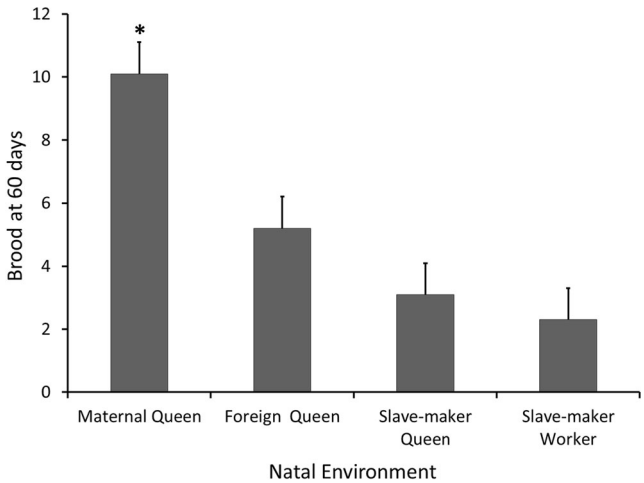

Fig. 3 Colonies containing workers and their maternal queen produced the most brood after 60 days ($F_{3,36}$=4.37, p=0.01). Values significantly different from the others are denoted with an *asterisk*

It is interesting that collective foraging and colony defense, although repeatable at the colony level, did not differ between rearing environments. As these behaviors are the emergent properties of the traits of individuals, we propose that these traits are either genetic in origin, or that the developmental mechanisms underlying these behaviors are be determined before eclosion (i.e., while workers were still larvae). Consistent with this hypothesis, the identity of the source colony from which the brood originated had a significant influence on the behavior of workers in all rearing environments (Table 1) and was included in two of our three top models predicting nest discovery (Table 2). Thus, these genetically determined or pre-eclosion developmental effects seem to supersede the influence of the post-eclosion social environment. Recent work on the rock ant *Temnothorax rugatulus* suggests that collective personalities differ consistently among colonies in the field and vary along geographic gradients (Bengston and Dornhaus 2014).

The amount of new brood produced was greatest in colonies containing a queen and her own brood. Given that the egg-laying capacities of *P. americanus* queens and workers is quite high (Blatrix and Herbers 2004), it is unlikely that the differences in brood produced after 60 days were a result of the different egg-laying capacities of the queens, but rather were driven by the brood care behavior of *T. longispinosus* workers. The decreased brood production in other colonies further suggests that a lack of relatedness between workers and queens can drive within-colony conflict and reduced productivity. The stability of cooperation in eusocial groups is predicted to require a reliable kin recognition mechanism, and thus, decreased colony recognition efficiency may impede

colony efficiency at behaviors such as brood care (Foitzik et al. 2007). Although worker ants might not be more aggressive towards each other per se, they could merely reduce or terminate the care of unrelated brood in their colony, whereas lower productivity in colonies containing a slave-making queen or worker may have been a result of egg killing by the workers (i.e., "slave rebellion"; Achenbach and Foitzik 2009; Achenbach et al. 2010; Pamminger et al. 2013).

Finally, we note that subtle differences in the rearing environment of *T. longispinosus* can beget wildly divergence colony-level syndrome structures. Depending on whether workers were reared by foreign conspecifics of slave-making workers, we observed different kinds of correlations across contexts. While we are reticent to argue any adaptive value for the observed syndrome structures, we are impressed by the notable parallels between syndromes at the individual and colony levels. A moderately sized number of empirical studies have shown that individual-level syndrome structures can vary dramatically as a consequence of individuals' rearing environments (e.g., fish: Bell and Sih 2007, crickets: DiRienzo et al. 2012, spiders: Sweeney et al. 2013; Royauté et al. 2014). The fact that we find similar developmental contingencies at the colony level in this system provides further evidence of the potential for cross-fertilization between the literatures on collective behavior and behavioral syndromes. Until now, we observe that the majority of the contributions have moved unidirectionally, from the collective behavior (or social insect) literature into the behavioral syndromes literature (Jeanson and Weidenmüller 2014; Jandt et al. 2014).

Conclusions

Here, we demonstrate that variation in the natal and early social environment can induce changes in both individual- and colony-level personalities in the ant *T. longispinosus*, and variation at one level of biological organization (i.e., individual aggressiveness) can shape variation in another interacting level of organization (i.e., collective nest discovery rate). Therefore, persistent social interactions early in life appear to be an essential element in the development of behaviors at multiple levels of biological organization in this system. Further, there seemed to be a strong genetic component (i.e., brood source identity) to both individual and collective behaviors. Consequently, it is perhaps impressive that any developmental effects of the early social environment were observed, given such a potentially strong genetic component. However, this result also corroborates other evidence for strong genetic components to both individual and collective traits in other social insects (i.e., division of labor, Stuart and Page 1991). Thus, we reason that future work should address how behavioral interactions between queens and workers may drive these behavioral changes. Lastly, we urge that more studies integrating ontogeny into the study of collective or colony-level personality are needed to discern the generalizability of our results.

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